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Behavioral Assays for Effects of Drilling Muds on Marine Animals

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ABSTRACT

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FOREWORD

The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining:

- the effects of toxic organic pollutants on individual species and communities of organisms;
- the effects of toxic organics on ecosystems processes and components;
- the significance of chemical carcinogens in the estuarine and marine environments.

Over the past decade, oil and gas drilling operations have resulted in millions of cubic feet of drilling muds and other byproducts being released into the marine environment. This report describes a project directed toward improving methods of determining the sublethal effects of drilling muds and components on scallops. The results of this study will be used to aid in the assessment of the potential impact of drilling mud discharge on marine organisms and ecosystems.

Henry F. Enos
Director
Environmental Research Laboratory
Gulf Breeze, Florida

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SECTION 1
OVERVIEW

The research funded by this grant had the following three objectives:

1. To develop an apparatus for remotely recording water pumping and valve movements by scallops in a laboratory environment.

2. To obtain data on the effects of whole drilling muds and selected mud components on filter feeding, using the appartaus developed in the first objective.

3. To adapt the apparatus developed in the first objective to permit recording these data from scallops held in field conditions, and to perform field tests of this Submersible Monitoring Unit (SMU).

These objectives are covered by the following three sections of this final report.

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R.K. Pegg and M. Winter, my primary research assistants on this project, made many significant contributions and willingly worked many hours beyond the call of duty. I also thank C. Ashton, G.S. Cripe, R. Cripe, R.L. Koelling, D. Morton, N. Rubenstein, J.M. Sprouse, D. Thompson, A.F. Tomlinson and E. Winter for technical suggestions and assistance, and N.L. Richards for loan of equipment. This work was supported by an Environmental Protection Agency Grant, R806121-01.

Section 2: An Electronic Method of Monitoring Valve Gaping and Water Pumping in Bivalved Molluscs

Filtering organic material from water is a common feeding strategy among marine and aquatic animals, and is the dominant strategy among primary consumers (Jørgenson, 1966). Because bivalved molluscs are one of the largest groups of filter feeding animals, numerous studies have been conducted on the mechanism of filter feeding and, particularly, on how various factors (e.g., salinity, size and concentration of particles, pollutants) influence filter feeding.

There exist several measurable components of filter feeding behavior in bivalves. First, the valves must be gaped for water to flow through the mantle cavity; consequently, the incurrent and excurrent aperture sizes influence the rate of water flow. Hence, the width the valves are gaped comprises one measure of filter feeding activity. Second, the water current passing through the mantle cavity is produced by the beating of cilia on the gill. Hence, the rate of water flow comprises another measure of filter feeding activity. Finally, eulamellibranch bivalves sort particulate matter collected on the incurrent gill surfaces, and form aggregates of uningestible particles called 'pseudofeces.' When sufficient pseudofeces are collected, the valves are rapidly closed, driving water out of the mantle cavity in all directions, including out the normally inhalent opening. Pseudofeces are carried out of the mantle cavity by this flow of water. Subsequently, the valves are opened and the cilia recommence beating (Yonge, 1947; Owen, 1966). Because the rate of pseudofeces ejection depends on the concentration of uningestible particulate matter in the water, the frequency of these

single rapid valve closures comprises a third measure of filter feeding activity in bivalves.

The number of techniques used for monitoring filter feeding in bivalves almost equals the number of studies completed. Water pumping by bivalved molluscs has been measured directly by inserting a rubber tube into the gill cavity or by attaching a rubber sleeve to the shell, and then by permitting the exhalent water to fill a calibrated measuring chamber (Galtsoff, 1926: Nelson, 1936; Loosanoff and Engle, 1947; Loosanoff and Tommers, 1948; Odlaug, 1948; Tammes and Dral, 1955; Loosanoff, 1961; Drinnan, 1964) or to drip onto a pair of electrodes (Davids, 1964). Other studies have measured water flow by observing movements of dye (Coughlan and Ansell, 1964) or various particles. For example, Hersh (1960) used cinematography to study the movement of particles, including tobacco mosaic virus. Still other researchers have studied water pumping indirectly by measuring the removal of suspended material by organisms. Haven and Morales-Alamo (1970) used a Coulter counter to compare the concentration of particles in water entering a chamber containing oysters to that leaving the chamber, while Tammes and Dral (1955) used a colorimeter for the same purpose. Chipman and Hopkins (1954) and Dorn (1976) measured clearing and uptake of radioactively labelled plankton and diatoms. Some of these latter studies also obtained information on retention of different sizes of particles.

Over the past 15 years, several researchers have used thermistor flow-meters in the field to measure the water flow produced by such filter feeding organisms as sponges (Reiswig, 1971; LaBarbera and Vogel, 1976), brachiopods (McCammon, 1965) and tunicates (Fiala-Medioni, 1978). Different

speeds of water flow take up different amounts of heat from a thermistor; hence, because the amount of electrical current passing through a thermistor changes with the thermistor's temperature, changes in the speed of water flow produce electrical changes. I have found no studies using thermistors to monitor bivalve water pumping.

Valve movements in bivalved molluscs have primarily been measured using simple mechanical linkages to pens on kymographs (Loosanoff and Engle, 1947; Loosanoff and Tommers, 1948; Butler et al., 1960; Lossanoff, 1961). More recently, Davenport (1979), Manley and Davenport (1979), and Davenport and Manley (1978) monitored shell movements using a strain gauge. The electrical current flowing through this type of component changes as its shape is physically changed. Scallop shell movements have also been monitored using strain gauges by researchers studying swimming as an escape response (Thomas and Gruffydd, 1971; Stephens and Boyle, 1978).

Many of the techniques described above are somewhat cumbersome and it is difficult to envision using them effectively in field conditions. Furthermore, in most cases the data are expressed in a visual format, thus requiring tedious conversion to achieve compatability with a magnetic tape recorder for storage or a computer for analysis.

Herein I describe an electronic method for monitoring filter feeding in bivalves. Shell movements are continuously monitored by an inductance tranducer, thus providing data on gape width, frequency of pseudofeces ejection and, in swimming species such as scallops, the frequency of swimming attempts. Water pumped from the mantle cavity is monitored by a thermistor positioned outside the exhalent aperture. Changes in shell gape

and water pumping rate result in changes in output voltages from their respective monitoring circuits. These voltages may be portrayed directly on a chart recorder or they may be converted to frequency-modulated audio signals and stored on magnetic tape for subsequent analysis.

This system of monitoring filter feeding in bivalves has been used in laboratory studies on the effects of pollutants on scallops (section 3), and has been modified to permit long-term recordings in the field (section 4).

APPARATUS

Monitoring Frame

The scallop (or other bivalve) is attached to a monitoring frame which holds the inductance tranducer, its mechanical linkage, and the thermistor probe (see Figure 2.1). The framework of the holding apparatus was constructed from 1.3 and 1.9 cm (I.D.) PVC pipe and fittings; the shapes of some fittings were modified for specific purposes. The rubber plunger portion of a PLASTIPAK 50cc syringe was used as a form-fitting seat for the scallop. After cleaning and drying the lower valve of the scallop (the right valve), a small wire loop was attached to this valve using an epoxy putty. A hook attached to one end of a threaded shaft passed through a hole in the seat and seat support, and a wing nut on the opposite end of the shaft permitted the scallop to be drawn down against the rubber seat.

A miniature nylon clevis was attached to the upper valve of the scallop (the left valve) using form-fitting epoxy putty and PERMABOND. This clevis permitted mechanical linkage of the upper valve to a loop of 3.6 kg-test nylon line. This line was routed around two 2.54 cm diameter nylon pulleys and its tension was set with a miniature brass turnbuckle.

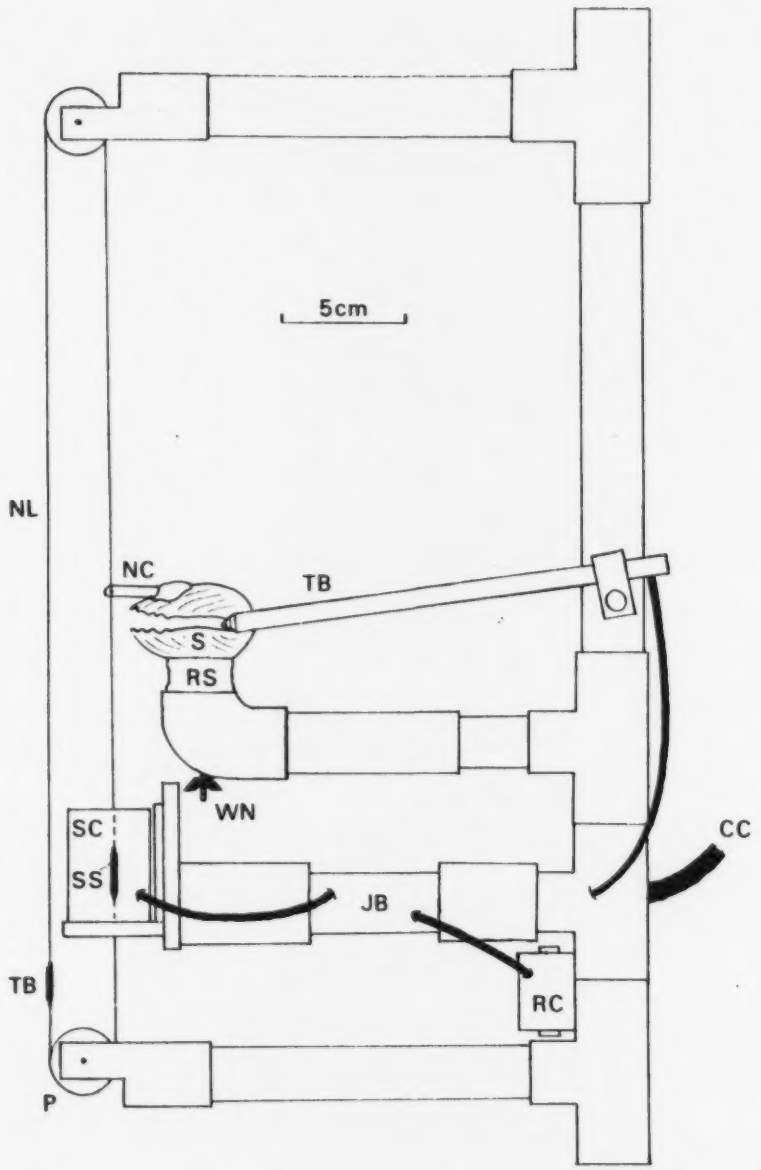


Figure 2.1. Frame used to hold a test scallop in position while monitoring its valve movements. (S=scallop's right valve; RS=rubber seat; WN=wing nut for tightening hold-down hook; NC=nylon clevis attachment; TB=adjustable boom with thermistor at its tip; RC=reference coil; JB=junction box; SC=sensor coil; SS=steel slug; NL=nylon line; TB=turnbuckle; P=pulley; CC=main connecting cable)

On the same side of the loop as the scallop, a 2.5 mm diameter, 20 mm long steel slug (section of galvanized 8 d nail), sheathed in plastic (heat-shrink tubing), was attached to the line using miniature brass eyebolts glued (PERMABOND) to the plastic sheath. As the scallop moved its upper valve, the steel slug travelled vertically through a plastic tube (9mm inside diameter) around which the sensor coil was wound. The coil was 20 mm long and had an estimated inductance of 437 microhenries with an air core. Movement of this slug changed the inductance of the coil. The brass eye-bolts had no appreciable effect on the coil's inductance. A second coil, identical to the first except for lacking the steel slug, was attached elsewhere on the monitoring frame and served as a reference coil.

Friction of the nylon pulleys was negligible. The clevis and steel slug (3 gm total) were located on one side of the loop of nylon line while the turnbuckle (1 gm) was located on the opposite side. Thus the scallop's hinge ligament had to lift the difference in these weights, 2 gm, in the process of opening the valves. The adductor must have been aided in its action by the weight of these objects. This is the identical situation encountered by scallops whose upper valves havevarious organisms attached to them in nature (e.g., solitary tunicates, Crepidula, chitons, serpulid worm tubes, hydrozoan colonies). I have frequently encountered scallops with more than 5 gm (total dry weight) of these organisms on the upper valve. Consequently, I conclude that this procedure for monitoring valve gaping exerts counterforces upon the movement of a scallop's valve that are less than those exerted by natural shell encrusting organisms.

The thermistor was embedded in the tip of an adjustable plastic boom. This boom was positioned so the sensor thermistor was located just

outside the large posterior exhalent aperture of the scallop. While some water flows out anterior to the hinge, in Argopecten irradians the greatest exhalent flow is posterior during normal filter feeding behavior; this is generally the case among other non-gastropod molluscs. Dakin (1909) reported similar observations for Pecten maximus. Water may be driven out anterior from the hinge during swimming (Gutsell, 1930).

Sensors were connected by waterproof cables to a section of the monitoring frame designated as the junction box. This area was waterproofed with wax after all connections were made. One large waterproof cable connected this portion of the frame to the associated circuitry and recording equipment located elsewhere.

Shell Movement Circuit

A sine-wave oscillator (1240 Hz @5.5 vac output) has its output connected to both the reference and sensor coils. Two full-wave rectifiers sample the voltage drops across the coils; when the coil inductances are equal, equal voltage drops occur across them. Since the rectifiers rectify the 1240 Hz voltages they sample in opposite polarities, their outputs cancel exactly and 0 vdc is fed to the recording device. As the steel slug moves into the field of the sensor coil, its inductance increases and the voltage drop across it increases. Since the reference coil's inductance remains unchanged, the output of the positive full-wave rectifier becomes greater than that of the negative full-wave rectifier; after their effects are combined at the output potentiometer, a positive voltage is fed to the recording device.

A schematic diagram of this circuit is shown in Figure 2.2. Portions

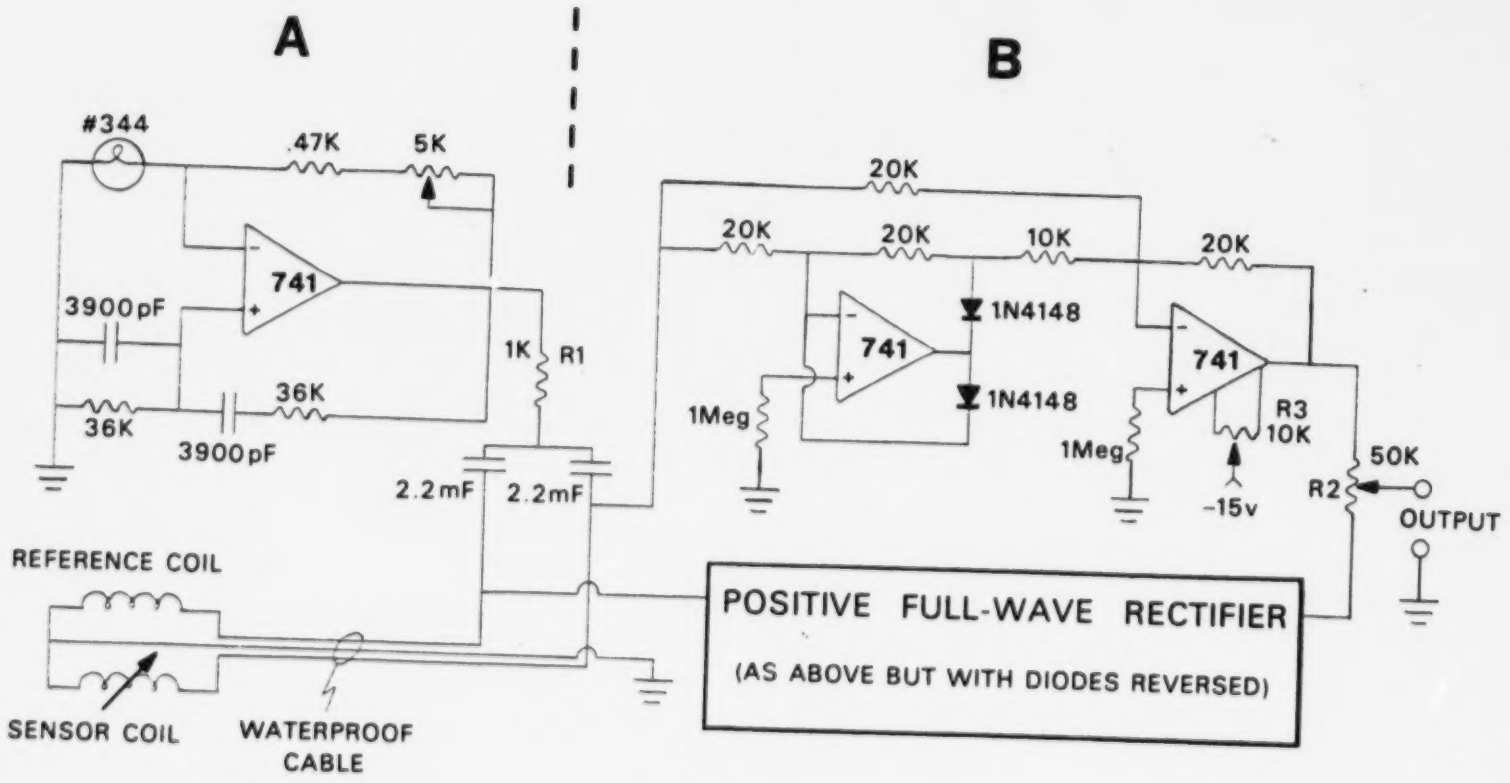


Figure 2.2. Schematic diagram of the shell movement circuit. It is comprised of an oscillator and sensor coils (A) and two full-wave rectifiers (B). All resistors have a .5-W rating and 5% tolerance, except for the 20K and 20K fixed resistors which have a 1% tolerance. The 741 integrated circuits are powered by ±15 vdc. The output may lead to a chart recorder or a voltage-to-frequency converter for recording on a magnetic tape.

of this circuit were modified from Jung (1974). Resistor R1 comprises the primary load for the oscillator output as the coils themselves comprise only a small load; the large capacitors (2.2 mfd) provide dc isolation from ground but little capacitive reactance for the oscillating signal. Potentiometer R2 permits zeroing the circuit output when the steel slug is out of the sensor coil. Only the schematic of the positive full-wave rectifier is shown in detail; the negative rectifier is identical except the polarities of its two diodes are reversed. An offset null adjust (R3) is used for the second amplifier of each rectifier to insure that zero output is produced when there is no input signal.

A log-log plot of the relationship between output voltage and slug insertion is approximately linear, and can be described by the following equation:

$$\log x = \frac{\log y + 2.31}{2.07}$$

where, x= slug insertion (mm)
y= output voltage (mv)

Sample traces of valve moments associated with normal filter feeding behavior are shown in Figures 2.4 and 2.6. Figure 2.3 shows a trace of the repeated rapid valve movements associated with normal filter feeding. Argopecten irradians.

Water Pumping Circuit

Initially, I attempted to develop a thermistor sensor circuit that would operate through a range of ambient current velocities while retaining sensitivity to the small currents produced by a pumping scallop. I modified

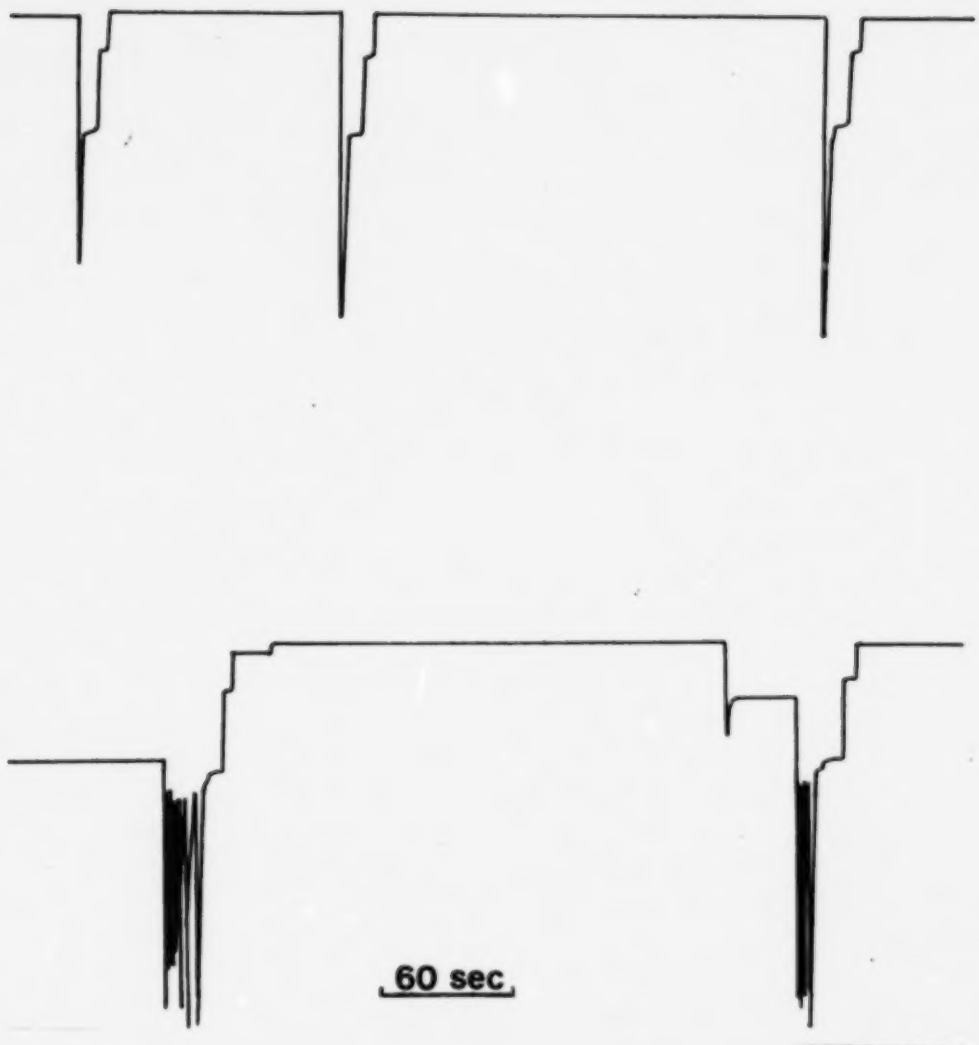


Figure 2.3. Rapid valve closures exhibited by a scallop, Argopecten irradians, during normal filter feeding behavior (top) and series of rapid valve closures associated with swimming attempts (bottom). Most swimming attempts lasted less than 5 seconds. The single rapid valve closures (top), which are frequently associated with a visible pseudofeces or feces ejection, are typically followed by a step-wise or "staircase" reopening of the shell. This situation was also noted by Thomas and Gruffydd (1971) for Pecten maximus.

the thermistor current meter described by LaBarbera and Vogel (1976:Figure 1A,B) so that both thermistors were exposed to water currents; one was positioned above the scallop and was exposed to the ambient water current only, while the other was positioned outside the posterior exhalant aperature of the scallop and was exposed to both the ambient water current and the exhalant water flow of the scallop. The circuit produced a dc voltage proportional to the difference in water flow over the two thermistors. I used YSI-44002A precision thermistors and modified the other components of LaBarbera and Vogel's bridge circuit appropriately.

This circuit worked adequately in calm water, as shown in Figure 2.4. However, the water pumping trace became somewhat irregular (noisy) when aeration was begun in the tank, and temporal changes in water pumping by the scallop became completely disguised by noise when even the slowest water current was induced by the paddles. These irregularities in the trace did not result from insufficient electrical current being fed through the thermistors. I suspect the trace irregularities were caused by irregular water flow patterns in the vicinity of the thermistor whose magnitudes were greater than the water current produced by the pumping scallop.

Since this problem would affect any thermistor regardless of the sophistication of its associated monitoring circuit, I developed a much simpler circuit for monitoring water pumping of scallops or other bivalves in calm water. The circuit is illustrated in Figure 2.5 and paired traces are shown in Figure 2.6. Only a single YSI-44002A thermistor is used. Resistor R1 sets the electrical current flow through the bridge, thus adjusting the sensitivity of the thermistor for different water temperature conditions. Resistor R2 balances the bridge for zero output when the scallop

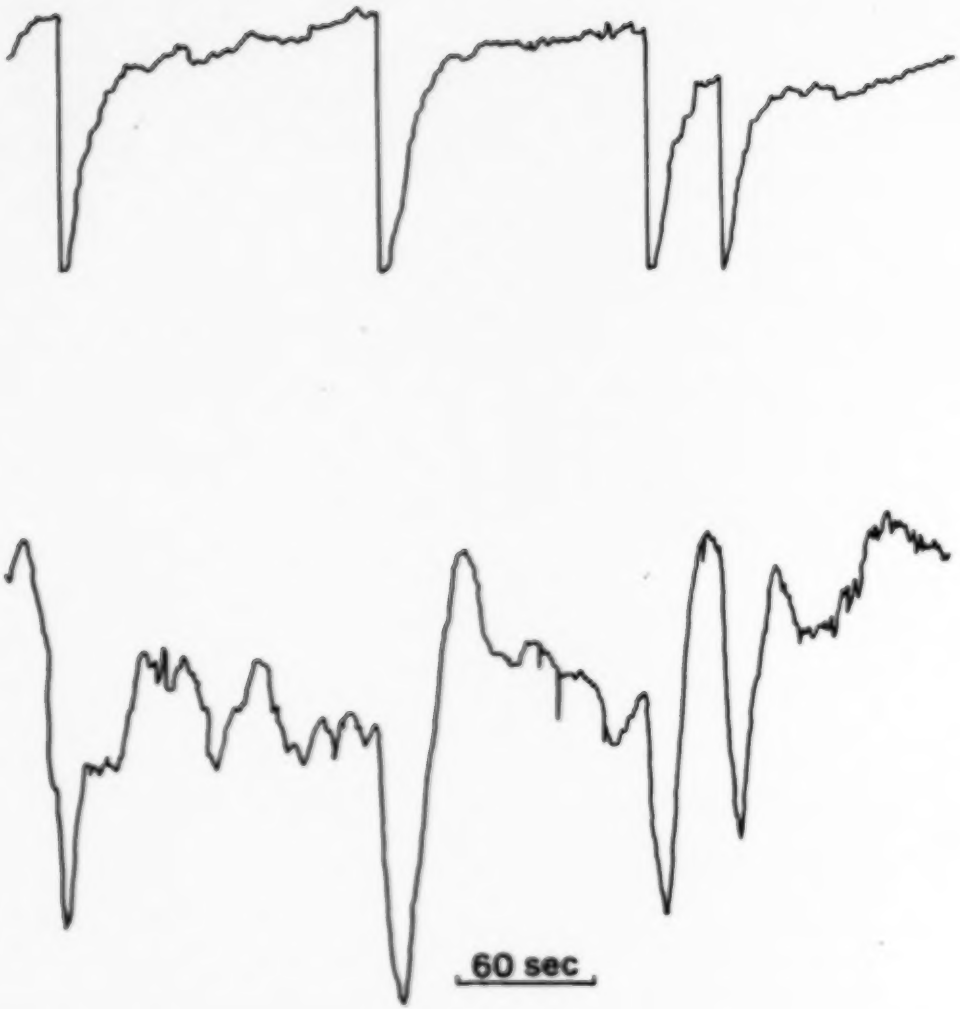


Figure 2.4. Paired traces of the water pumped by a scallop (bottom) and the simultaneous shell movements of the same scallop (top), obtained using a modified version of LaBarbera and Vogel's (1976) thermistor current meter. Traces were recorded in calm water (no aeration, paddles stopped).

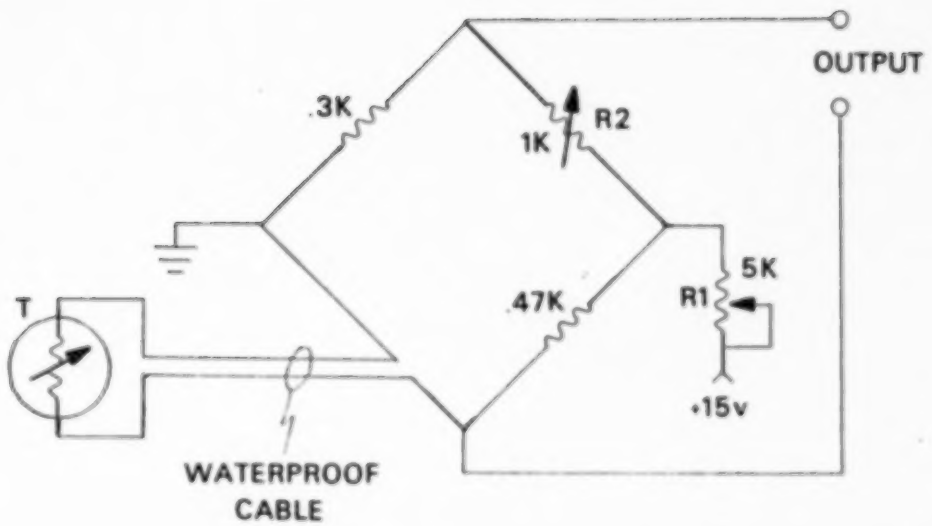


Figure 2.5. A simple single-thermistor sensor circuit for monitoring water pumping by a scallop or other filter feeding organism. Paired traces obtained using this circuit are shown in Figure 2.6.

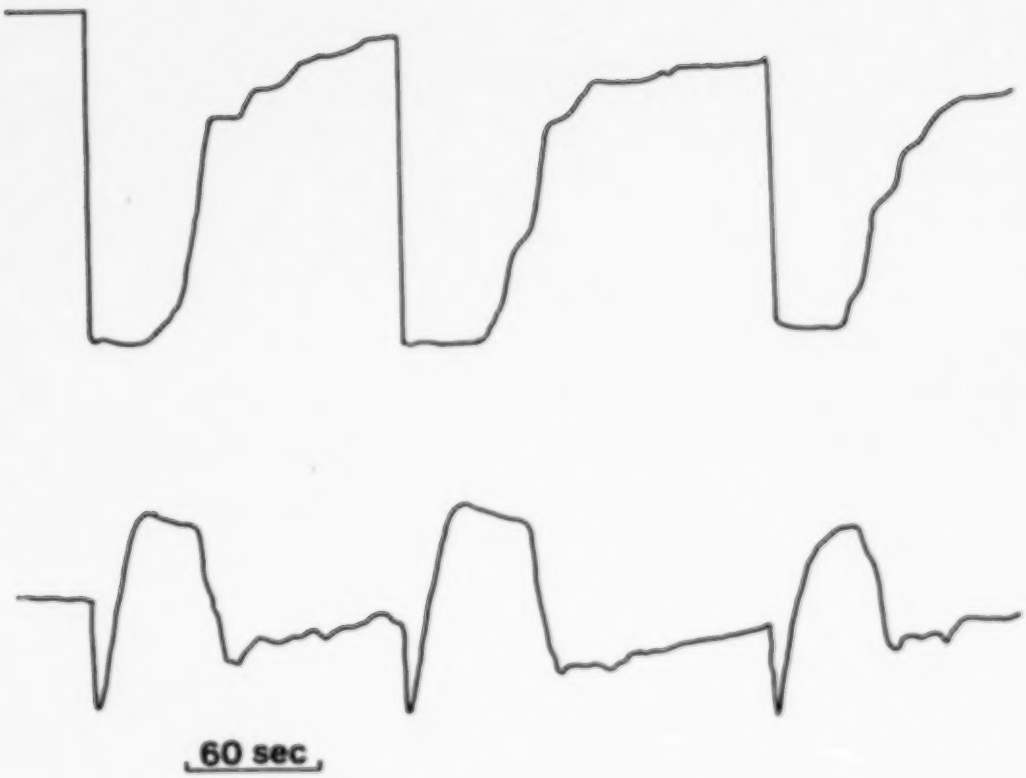


Figure 2.6. Paired traces of water pumped by a scallop (bottom) and the simultaneous shell movements of the same scallop (top), obtained using the simple thermistor circuit shown in Figure 2.5. Interestingly, these traces showed a rapid increase in water pumping after a rapid valve closure but a much slower reopening of the valves. Because the valves rarely close completely, this effect may be related to the increased velocity of water flowing through a smaller aperture.

is not pumping. Neither of these circuits is temperature compensated and neither measure absolute current flow. These features are not considered problems because the circuits would necessarily be used in a controlled laboratory environment and researchers are usually most interested in changes in water pumping, or in measuring pumping as percent of normal response.

Voltage-to-Frequency Converter

For storage of data from the shell movement circuit on magnetic tape, we employed the basic circuit described by LaBarbera and Vogel (1976:Figure 1C). However, we replaced their 15K resistor linking the input to pin 7 of the 555 timer with a 23K resistor. Also, we deliberately adjusted the output potentiometer of the shell movement circuit (R2) for about 2.3 vdc output when the slug was out of the sensor coil so the timer would always have some voltage biasing this pin. (We adjusted R2 for 0 vdc when the output was fed directly to a chart recorder.) With this bias, the timer pulses at about 1 KHz. As the slug enters the coil, the pin 7 input increases as does the timer pulse frequency. Timer output is connected directly to the input of a tape recorder.

Frequency-to-Voltage Converter

To retrieve the data from the magnetic tape, we modified the circuit described by LaBarbera and Vogel (1976:Figure 1D). Our circuit is shown in Figure 2.7. In order to reduce extraneous noise, magnetic tape casettes were played back with the best quality tape recorder available.(We used a WOLLANSAK 2551.)

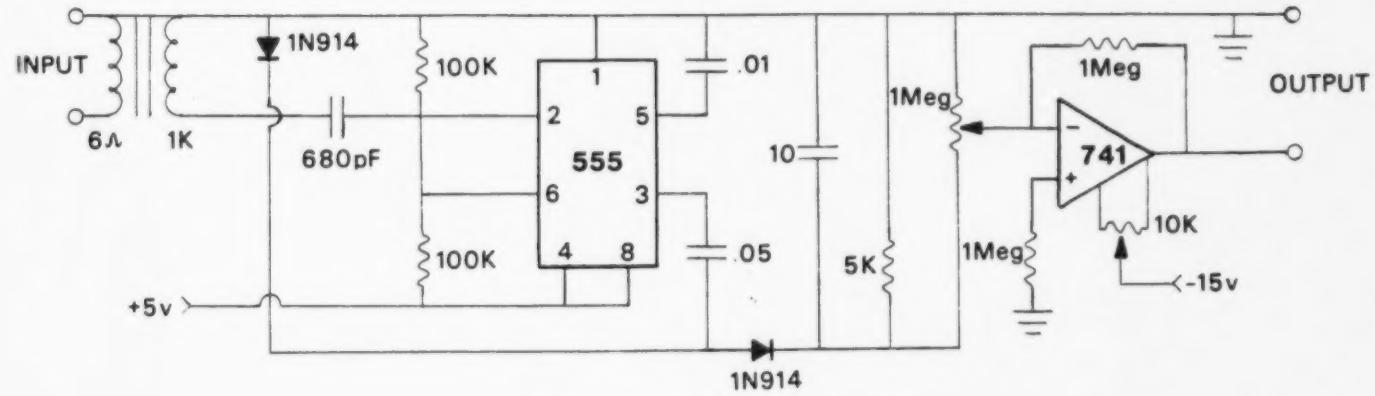


Figure 2.7. Schematic diagram of the frequency-to-voltage converter I modified from LaBarbera and Vogel (1976). The 741 integrated circuit is powered by ±15 vdc.

Section 3: Effects of Drilling Muds and Selected Mud Components on the Shell Movements of Scallops.

During the process of drilling for oil in the marine environment, quanities of drilling muds and drilled-up solids (cuttings) are discharged into the surrounding water. Drilling muds are chemically complex and contain both water soluble and water insoluble components. A visible plume containing drilling mud and cuttings is produced by the prevailing water currents. Thus this material is distributed in the water column and on the bottom in the area around the drilling rig (McGuire, 1975; Monaghan et al., 1976; Richards, 1977).

Few critical studies have been directed toward evaluating the influence of whole mud or its components on the environment. Effects of whole drilling mud or its important components have been documented on the composition of benthic communities (Tagatz et al., 1977, 1978a, 1979b; Tagatz and Tobia, 1978; Cantelmo and Rao, 1978a,b; Cantelmo et al., 1979). Thompson and Bright (1977) studied the sediment clearing response of hermatypic coral polyps. Polyps were unable to remove whole drilling muds but could remove equal doses of barite, aquagel and calcium carbonate. Studies on other species and other behaviors are lacking.

The commercial scallop, Argopecten irradians,was chosen for study of the effects of drilling muds for several reasons. First, most of what comprises drilling mud consists of fine particulate matter which remains in suspension for varying amounts of time. Hence, it would seem that filter feeding organisms would be the first to come in direct contact with this material. Second, scallops can swim and, according to some biologists, they are supposedly capable of migration. Although the migration question

is far from answered (see Baird, 1966; Hartnoll, 1967), scallops do swim in response to predators (e.g., Thomas and Gruffydd, 1971; Stephens and Boyle, 1978). Since Ensis and other normally infaunal bivalves will sometimes swim in response to degraded water quality (Ansell, 1969), it was anticipated that scallops, for which swimming is a much more common behavior, might swim and be carried horizontally by ambient water currents when exposed to poor water quality. Similar strategies are involved in the migrations of eel elvers (Creutzberg, 1963), portunid crabs (Venema and Creutzberg, 1973) and shrimp (Hughes, 1969), and may occur in other molluscs (Hamilton and Ambrose, 1975; Schuhmacher, 1973). Third, scallops are found in areas of the Gulf of Mexico where drilling occurs. The bay scallop, Argopecten irradians, was once very common in Mobile Bay, Alabama, and the Pensacola estuary, though it is less so now. (The mud sample tested in this study came from a drilling rig at the mouth of Mobile Bay.) The calico scallop, Argopecten gibbus, is found further offshore in the Gulf of Mexico where drilling operations occur more frequently. Finally, scallops are commercially important. The fishery for bay scallops has markedly declined over the past 10 years in Florida and the calico scallop fishery is currently following a sharp downward trend (Florida Landings).

This study was designed to measure the effects of different doses of whole drilling mud and of two major particulate components on the filter feeding responses of the bay scallop, Argopecten irradians. These effects were compared to those caused by equal doses of calcium carbonate and by clean but unfiltered sea water (the control). Because the valve movements of a bivalve have not been analyzed in detail before, it was not known

which parameters would clearly reveal responses to particulate matter and which ones would not. Therefore, this study also comprises an analysis of the relative reliability of several different valve movement parameters as indicators of response to particulate material.

METHODS AND MATERIALS

Valve movements of scallops were monitored using the frame and electronic circuitry described in Section 2. Water pumping was not measured because the necessary monitoring circuit had not yet been perfected at the time these studies were begun. Two scallops mounted on individual monitoring frames were maintained in each of two tanks while their behavior was recorded. Each tank contained 126 liters of unfiltered ambient sea water. The outputs of the four valve gape monitoring circuits were recorded on two OMNISCRIBE B-5000 dual-channel chart recorders (Houston Instrument Co.), operating at 2.5 cm/min.

The circular test tanks were custom made following the basic design of Creutzberg (1963) and Venema and Creutzberg (1973). The inside diameter of the tanks was 61 cm and the inside diameter of the paddle-containing cylinder in the center was 30 cm. Each tank possessed a cone-shaped bottom; a centered drain lead to an air-lift pipe which ran up the outside wall of the tank and into the outer portion. The cone shape and air-lift permitted continuous recycling of the tank contents, thus keeping the particulate materials in suspension. The paddles in the two tanks were driven with a single Craftsman (.5 hp, 115 vac) variable-speed motor. A pulley reduction array, a variable transformer and adjustable-pitch paddles permitted accurate control of current speed. The current speed in the outer portion of the tank (where the scallops were located) was 5.85 cm/sec during all tests

described here. Two fluorescent bulbs were centered over each tank. The tanks and all associated apparatus were housed in a small room from which all people were excluded during test runs.

Adult scallops (shell height ≥45 mm) were collected periodically from shallow grassflats in East Bay, Panama City, Florida. They were transported to 10 cm deep outdoor runways at the Environmental Protection Agency Laboratory on Santa Rosa Island. There they were exposed to natural photoperiod and flow-through unfiltered seawater from Santa Rosa Sound. Temperature and salinity were ambient. This same water, to which the animals were already adapted, was also used in the two tanks during tests.

A 20-liter sample of whole drilling mud was obtained at 1130 hrs. on August 7, 1979, from a Mobil Oil Co. exploratory drilling rig located near the mouth of Mobile Bay, Alabama. The temperature of the mud entering the mud room was about 77°C but the "mud engineer" estimated that at the hole's bottom (then 5850 meters), the mud's temperature was over 175°C. The mud sample was transported to the Environmental Protection Agency Laboratory in Gulf Breeze, Florida, where it was stored temporarily at 13°C, and later at 3°C. Samples were taken for testing after thoroughly mixing the mud in the storage container. The mud had a smell resembling diesel fuel, a material that is sometimes added to mud on drilling rigs.

Barite (barium sulphate; produce name IMCO-BAR) was obtained from the Mobil rig on the same day. Barite (density, 4.25) is the major drilling mud additive by weight. Another major component of drilling mud, lignosulfonate [sic] was obtained from IMCO Services in Mobile (produce name IMCO RD-111), the distributor that was supplying mud components to the same rig from which the mud sample and barite were obtained. The distributor claimed this type

of lignosulfonate contained sodium chromate, although their product literature did not mention this. Powdered calcium carbonate (analytical grade; density 2.75) was obtained from Mallinckrodt Chemical Works. When aliquots of the mud sample were dried by heating, the average residue concentration was measured to be 440 gm/l. This figure was used to determine the volume of whole drilling mud needed for standard concentrations in the test tanks. Concentrations of barite, lignosulfonate and calcium carbonate were computed on a weight basis.

Tests of whole mud, barite, lignosulphonate, calcium carbonate and undosed sea water were conducted from August 15, 1979, to February 20, 1980. Over this period, the salinity of the water in the test tanks ranged from 18 to 26 ppt and the temperature ranged from 9 to 28°C. For a given concentration of test material (e.g., 400 ppm), 9 to 11 (usually 10) scallops were tested with each of the four particulate materials, and with the seawater control, following a rotating sequence. Those few scallops which exhibited fewer than three rapid valve closures during the pre-dose period (see below) were discarded from the analysis. Once all scallops were tested at one concentration, tests at the next concentration were begun.

Preliminary observations of scallops exposed to undosed seawater revealed considerable variation in valve movement patterns among animals, with much less variation for individual animals over time. For this reason, I compared the responses of individuals before and after exposure to a test material. After each scallop was attached to its monitoring frame, it was suspended in a tank containing undosed seawater, with the tank paddles running. Twenty-five minutes after all four scallops were introduced to their tanks (a period of acclimation), a 54-minute period of monitoring was

begun; this was designated the pre-dose period. At the end of this period, known amounts of whole mud, barite, lignosulfonate or calcium carbonate were introduced to the appropriate tanks; nothing was added for the seawater control tests but these scallops were equally disturbed by the investigator's movements in the room. Preliminary observations indicated that 5 minutes was sufficient time for undosed scallops to return to normal behavior after departure of the investigator from the room. Therefore, 5 minutes after the introduction of test materials a second 54-minute period of monitoring was begun; this was designated the post-dose period.

The following data were recorded for both the pre- and post-dose periods of each scallop tested:

1. The instantaneous shell gape at 2 min. intervals. The average gape distance for each period (n=27) was later computed. The change in average gape distance between pre-and post-dose periods was taken as the measure of the response of the animal.
2. The change in gape distance resulting from each RVC, the cumulative total for the magnitude of all RVC's and the number of RVC's. An RVC was defined as a closure resulting in a change in gape width that was completed within 2 seconds. In practice, most closures were completed in less than 1 sec and we were able to detect valve closures as small as .3 mm. The magnitude of the average RVC for each period was later computed, and 'major' RVC's (see below) were identified from all RVC's that occurred.

In summary, effects are presented in terms of change in shell gape, change in average magnitude of all RVC's, change in the cumulative total of all RVC's, change in the number of all RVC's, and change in the number of

'major' RVC's. All pre- and post-dose comparisons were made using the Paired t-test, (Zar, 1974) with .05 as the criterion level for significance.

RESULTS

Figure 3.1 shows the relationship between the average change in gape distance of the valves for five concentrations of each of the four materials tested, and for their corresponding seawater controls. None of the relationships (including whole mud) exhibit clear trends and there are only a few scattered points which are significant. For example, 400 ppm whole mud caused a significant 3.23 mm decrease in gape distance and 50 ppm lignosulphonate caused a significant 2.47 mm increase. The change in valve gape observed in the seawater controls for the 100 ppm test group was the only time seawater produced a significant effect for any valve movement parameter in any of the test groups. These results indicate that changes in gape distance of the valves, at least with the materials and concentrations tested, are not a reliable measure of response.

Figure 3.2 shows the relationship between the average change in magnitude of all RVC's and concentrations of the materials tested. Again, none of the relationships (including whole mud) exhibit clear trends and there are only a few points which are significant. For example, 400 ppm whole mud caused a 1.4 mm increase in closure magnitude while 200 ppm of lignosulphonate and barite caused .85 mm and .95 mm increases, respectively. These results indicate that changes in the magnitude of all RVC's, at least with the materials and concentrations tested, are not a reliable measure of response.

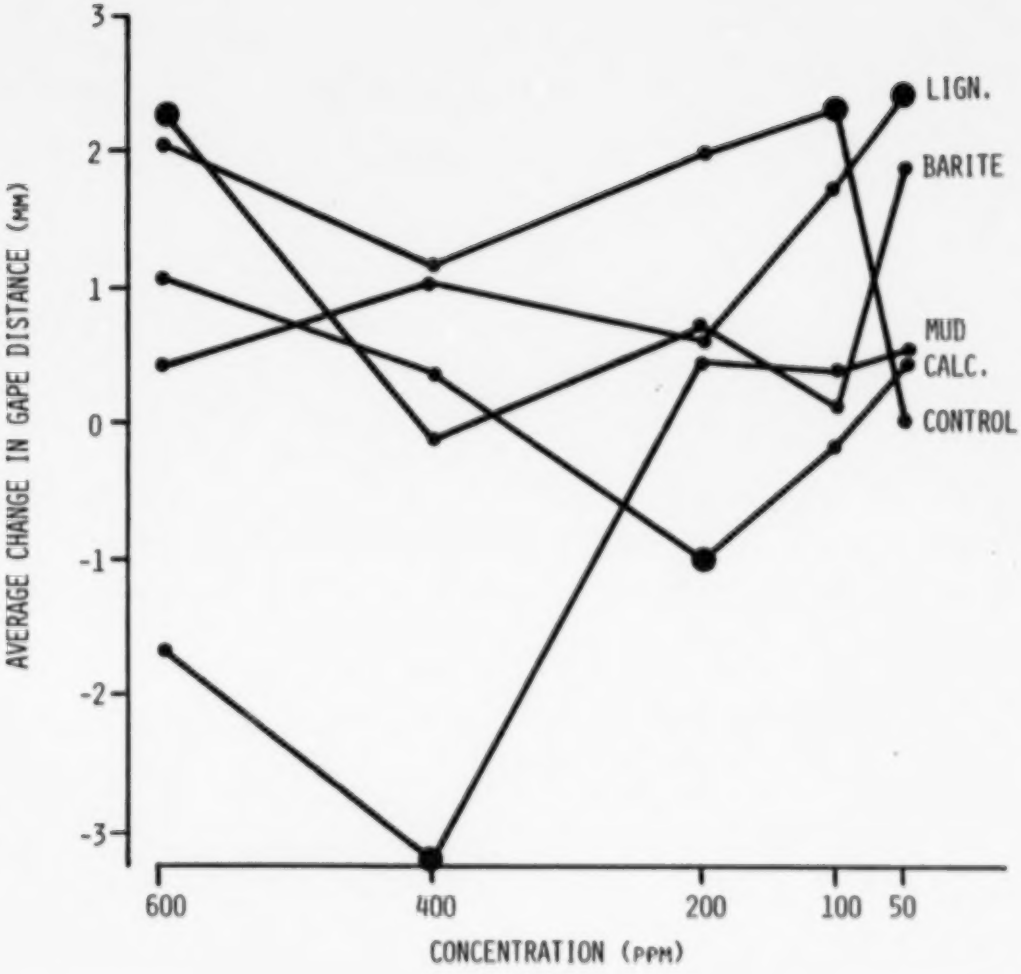


Figure 3.1. Relationship between the concentrations of five materials and the average change they caused in the distance the valves of scallops are gaped (open). The larger dots indicate points that are significantly different from zero.

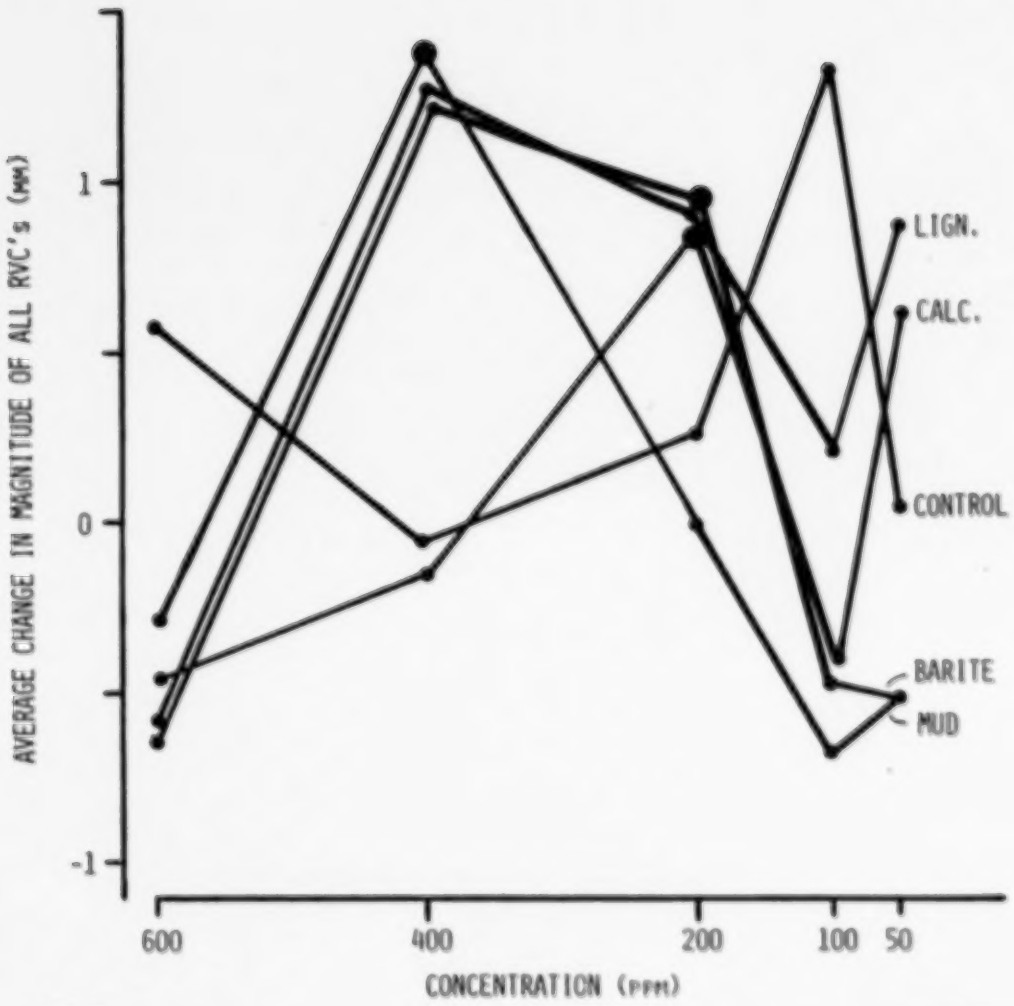


Figure 3.2. Relationship between the concentrations of five materials and the average change they caused in the magnitude of Rapid Valve Closures (RVC's). Larger dots indicate points significantly different from zero.

Figure 3.3 shows the relationship between the average change in the number of all RVC's and concentrations of the materials tested. All of the particulate materials showed significant effects for at least two concentrations. Barite showed significant effects at all concentrations, even at 50 ppm, where lignosulphonate also showed a significant effect. Although the whole mud results suggested a crude relationship between dose and response, there were no clear trends for any of these particulates. The sea water control produced uniformly low and insignificant effects. These results indicate that changes in the number of all RVC's, at least with the materials and concentrations tested, are only a marginally reliable measure of response.

It is apparent from observing traces of the valve movements of individual scallops that a frequency distribution of the magnitude of RVC's is often bimodal; over a period of time, scallops tend to exhibit either large magnitude or low magnitude RVC's. Examples from two scallops are shown in Figure 3.4. Because large magnitude RVC's would seem more likely to be associated with a pseudofeces ejection, it would be desirable to analyze responses to particulates on the basis of changes in large magnitude RVC's, or as I have termed them here, 'major' RVC's. The initial problem with this approach is defining what constitutes a major RVC. Any such criterion needs to be indexed for each specific animal; as illustrated in Figure 3.4, most major RVC's for one scallop are in the 8 to 12 mm range, while most major RVC's for the other scallop are in the 5 to 9 mm range.

My solution to this problem is to define a major RVC for a scallop as any RVC greater than or equal in magnitude to the average RVC exhibited by the scallop during the pre-dose period. This criterion is then applied to both the pre- and post-dose RVC's to identify those RVC's which were

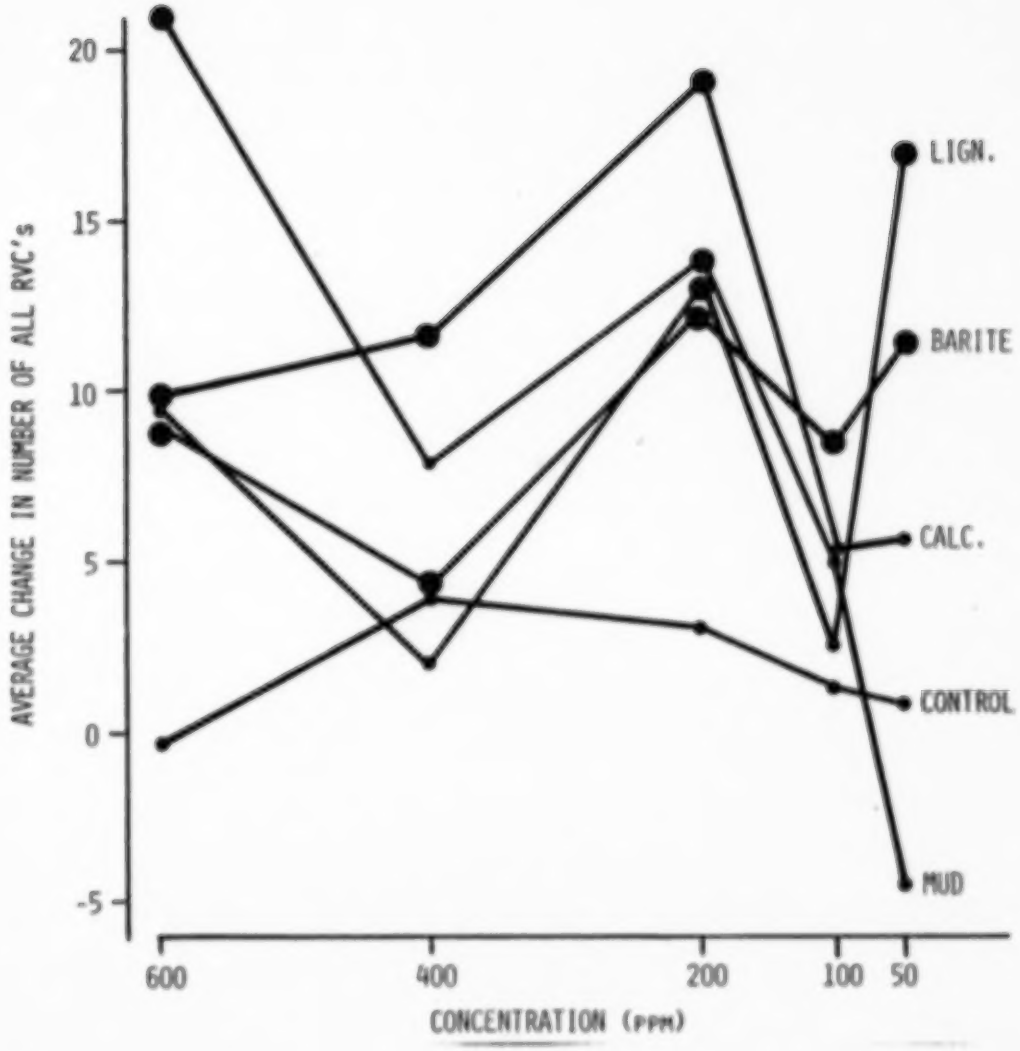


Figure 3.3. Relationship between the concentrations of five materials and the average change they caused in the number of all RVC's. Larger dots indicate points significantly different from zero.

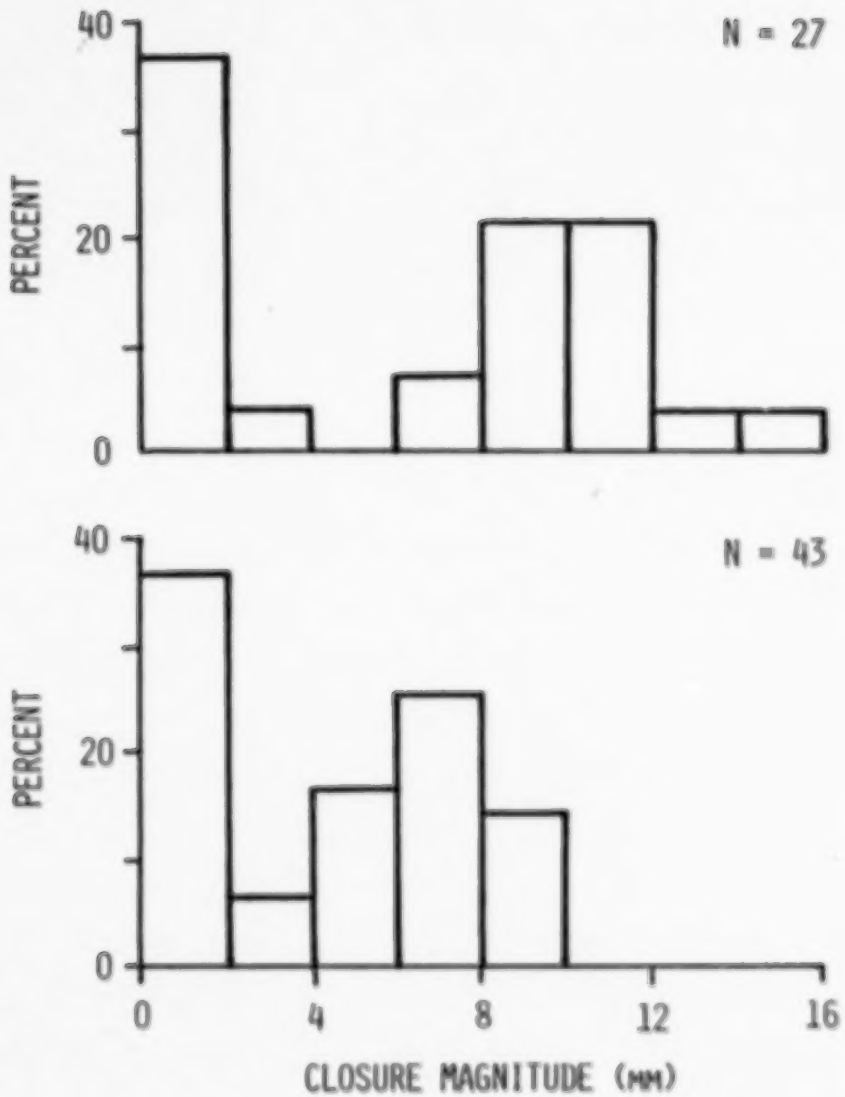


Figure 3.4. Frequency distributions for the magnitude of all RVC's exhibited by each of two scallops during 54-minute pre-dose monitoring periods.

major for that animal. Figure 3.5 shows the relationship between concentration and average change in number of major RVC's for whole mud and the paired sea water controls. A clear trend is evident in the dose-response relationship for whole mud. Concentrations of 600 and 400 ppm caused increases of 9.3 and 7.4 major RVC's, respectively; lower concentrations of mud did not produce significant effects. The sea water control showed no significant effects for any test group. These results indicate that the change in number of major RVC's is a reliable indicator of response to whole drilling mud.

Figure 3.6 shows the relationship between concentration and average change in number of major RVC's for barite, lignosulphonate and calcium carbonate. All three of these particulates exhibited a peculiar dose-response relationship, although only a few scattered points were significant. An increase in major RVC's was seen at 200 ppm for all three materials. Calcium carbonate and barite produced equally low effects at lower concentrations (100 ppm, 50 ppm), but 50 ppm lignosulphonate produced a greater increase in number of major RVC's than any of the materials at any of the concentrations tested.

It is also apparent from observing traces of the valve movements of individual scallops during post-dose periods that some individuals responded by making few but large-magnitude RVC's while others responded by making many but small-magnitude RVC's. Hence it would be desirable to analyze responses on the basis of total magnitude of all closures during a period, or as I have termed it here, 'cumulative magnitude' of all RVC's.

Figure 3.7 shows the relationship between concentration and the average change in cumulative magnitude of all RVC's for whole mud and the

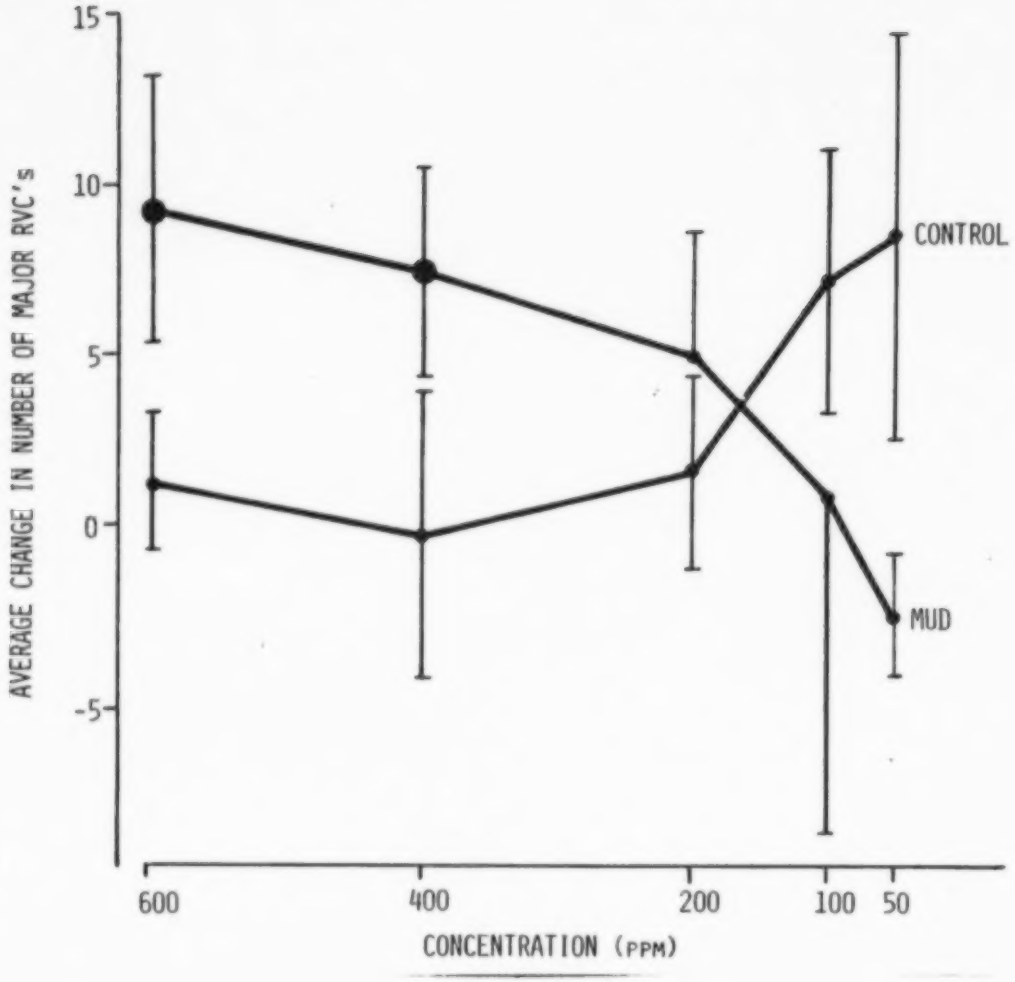


Figure 3.5. Relationship between the concentration of whole mud and the paired sea water controls, and the average change they caused in the number of major RVC's. Larger dots indicate points significantly different from zero. Bars are standard errors.

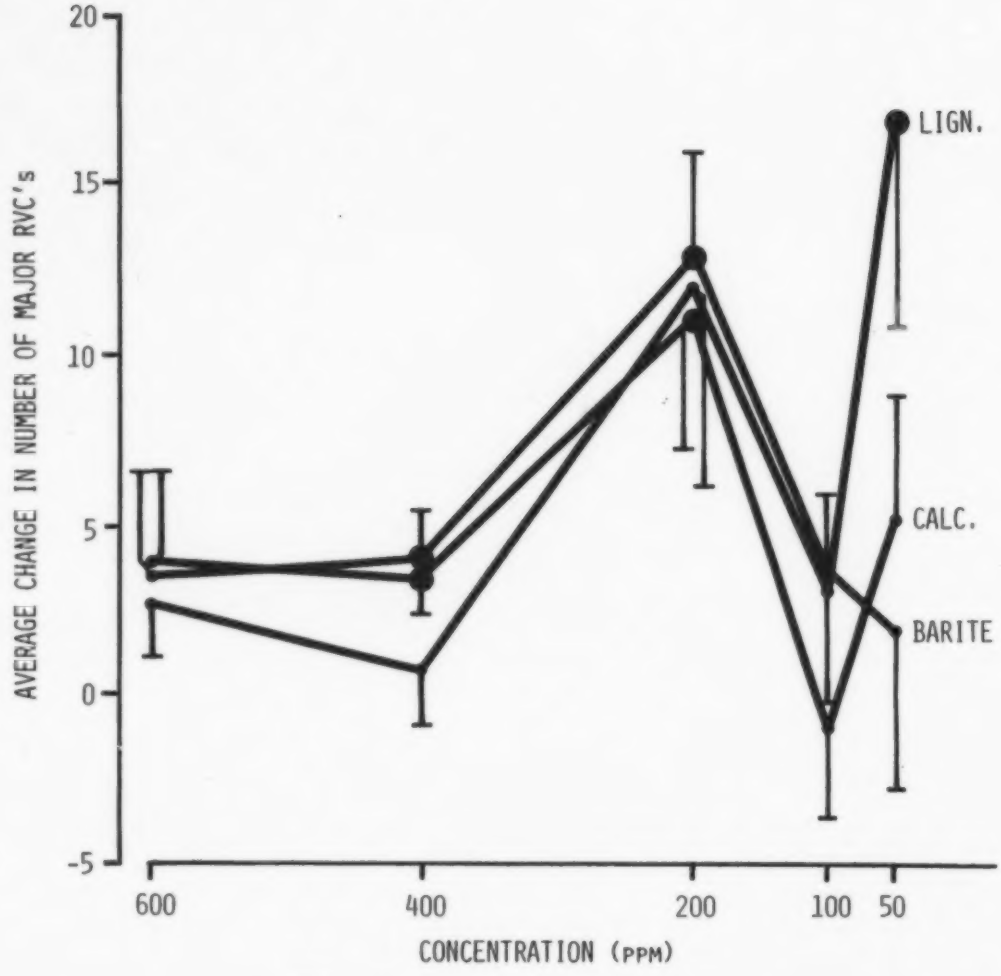


Figure 3.6. Relationship between the concentrations of barite, lignosulphonate and calcium carbonate, and the average change they caused in the number of major RVC's. Larger dots indicate points significantly different from zero. Bars are standard errors.

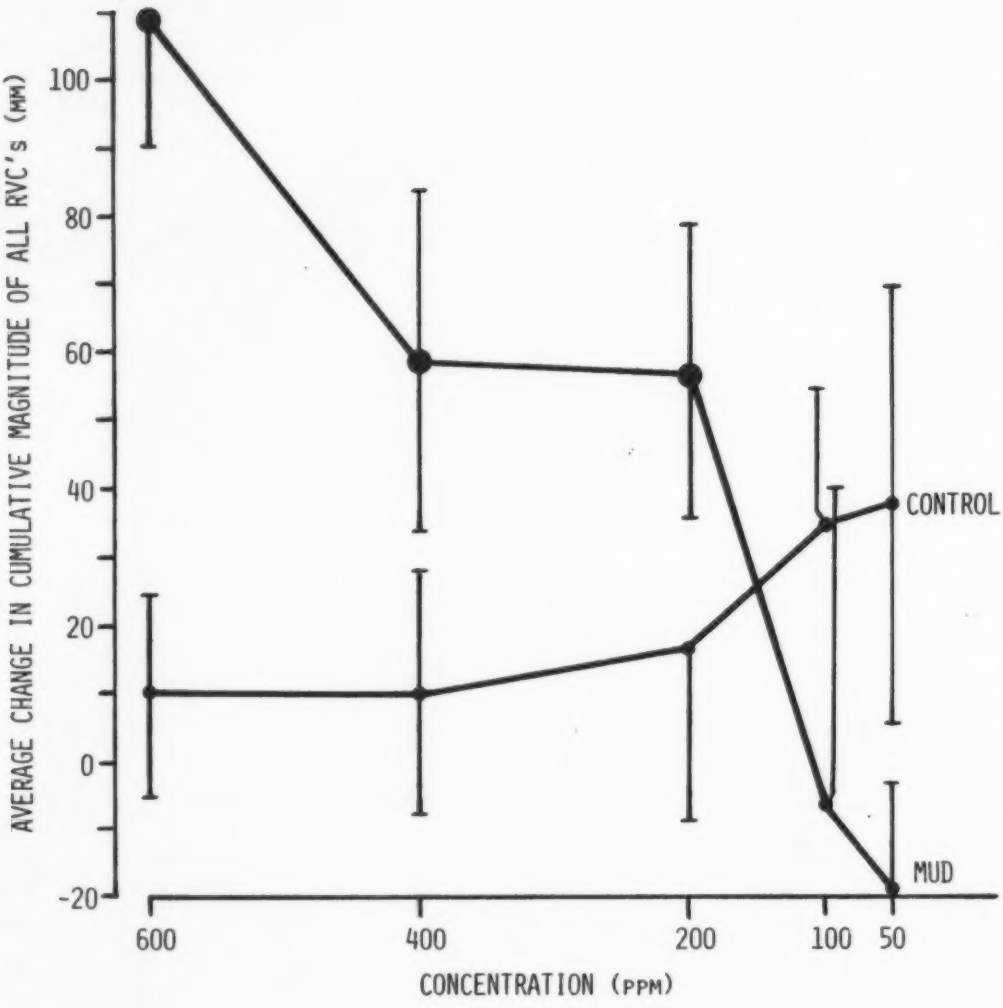


Figure 3.7. Relationship between the concentration of whole mud and the paired sea water controls, and the average change they caused in the cumulative magnitude of all RVC's. Larger dots indicate points significantly different from zero. Bars are standard errors.

paired sea water controls. A clear trend is evident in the dose-response relationship for whole mud. Concentrations of 600,400 and 200 ppm caused increases of 109,58 and 55 mm of total RVC's respectively; lower concentrations of mud did not produce significant effects. The sea water control showed no significant effects for any test group. These results indicate that the change in cumulative magnitude of all RVC's is a reliable indicator of response to whole drilling mud.

Figure 3.8 shows the relationship between concentration and the average change in cumulative magnitude of all RVC's for barite, lignosulphonate and calcium carbonate. As with the data in Figure 3.6, only a few scattered points were significant but an increase in cumulative closures was again seen at 200 ppm for all three materials. Calcium carbonate and barite produced equally low effects at lower concentrations, but 50 ppm lignosulphonate again produced a greater increase in total RVC's than any of the materials at any of the concentrations tested.

DISCUSSION

Of the five valve movement parameters analyzed, two are not reliable indicators of response (change in valve gape, change in RVC magnitude) and a third parameter (change in number of all RVC's) is only a marginally reliable indicator of responsiveness to whole drilling mud. The last two parameters (change in number of major RVC's, change in cumulative RVC magnitude) appear to be reliable indicators of responsiveness to whole mud, presumably due to the fact that these parameters are least influenced by the natural variations exhibited by scallops. The major-RVC parameter

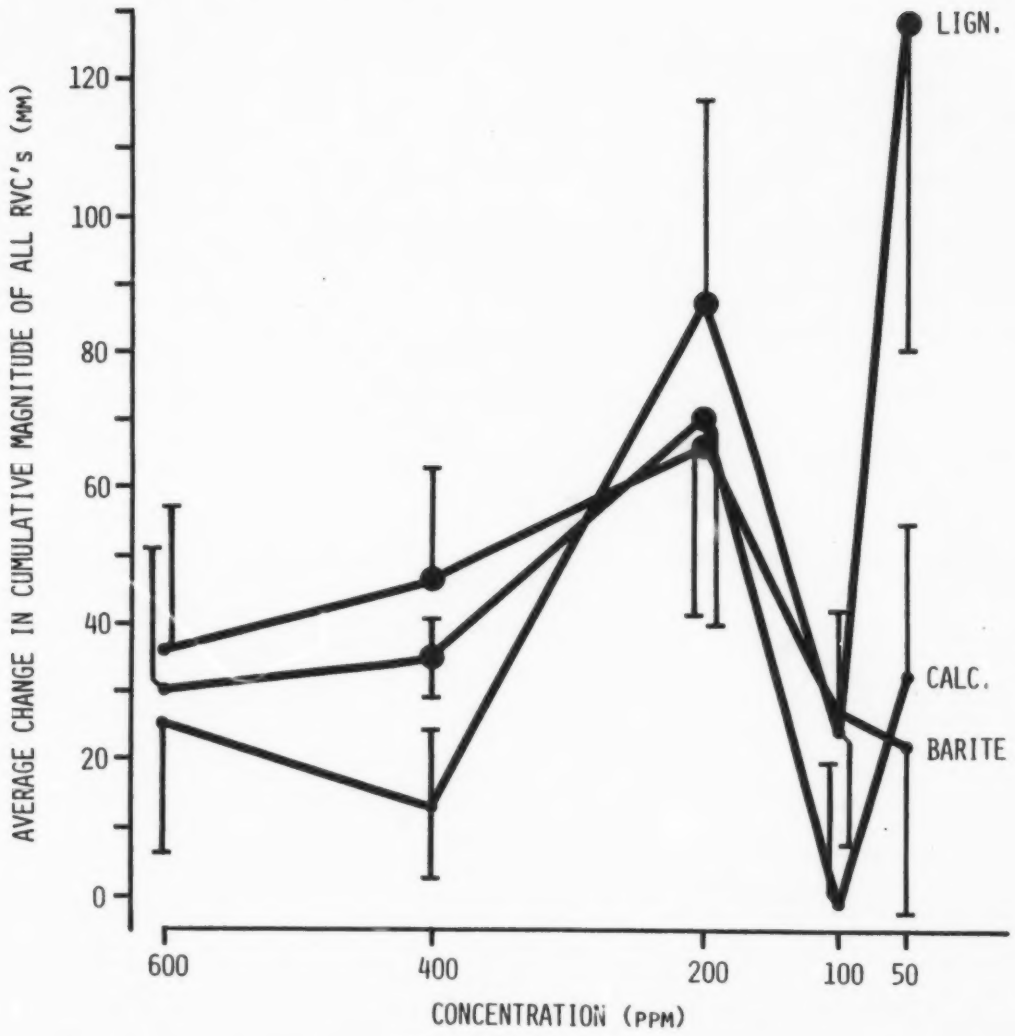


Figure 3.8. Relationship between the concentration of barite, lignosulphonate and calcium carbonate, and the average change they caused in the cumulative magnitude of all RVC's. Larger dots indicate points significantly different from zero. Bars are standard errors.

permits defining what is a 'major' or large closure for each scallop while the cumulative-RVC magnitude parameter ignores difference in response patterns. Using these last two valve movement parameters, certain conclusions can be drawn about the effects of whole drilling mud and the other particulate materials on this behavior.

The first conclusion is that whole drilling mud significantly increases the number of major RVC's above 400 ppm and significantly increases the cumulative magnitude of RVC's above 200 ppm. These results almost certainly reflect the increased number of pseudofeces ejections at these doses. There was a clear dose-response relationship for both parameters for whole mud (Figures 3.5, 3.7).

The second conclusion is that a clear dose-response relationship does not exist for the three other particulates tested (barite, lignosulphonate and calcium carbonate), though as a group they all exhibited approximately the same pattern of effect (Figures 3.6, 3.8). A greater effect was observed for these three materials at 200 ppm than at either of the two higher concentrations, a situation not observed for whole mud, which was tested simultaneously. Presuming this phenomenon is real, there seems to be only one reasonable explanation: for "pure" insoluble particulates, there is a concentration (e.g., 300 ppm) above which scallops tend to "give up trying" to clear the gills via pseudofeces ejections, but some factor associated with the complex composition of whole drilling mud over-rides this tendency. The reality of this phenomenon could be tested via an acute toxicity study because scallops which no longer keep their gills clean of particulates are likely to experience higher mortality.

Of the three particulates whose patterns of effect are illustrated in Figures 3.6 and 3.8, barite and calcium carbonate appeared to have the most similar patterns; lignosulphonate deviated the most from the basic pattern at 50 ppm, the lowest concentration tested. A reasonable explanation for this lies in the nature of the particles involved. Microscopic examination showed that the barite sample consisted of homogenous approximately-spherical particles in the 3 to 5 um size range. The powdered calcium carbonate also consisted of homogenous approximately-spherical particles in the 5 to 13 um size range. Lignosulphonate, however, consisted of much larger particles of two types; I estimate that about 10% of the particles were spheres from 17 to 38 um in diameter while 90% of the particles had irregular oblong shapes with dimensions ranging from 60 to 110 um in width and 89 to 460 um in length.

An additional facet of the similarity in patterns of effect for these three particulates is a consideration of their relative densities. Barite has a density of from 4.2 to 4.7 (IMCO Services product data) while calcium carbonate's density is about 2.75. Because the concentrations of particulates were prepared on a weight (ug/l) basis, this means that at any one concentration (ppm), there were many more calcium carbonate particles than barite particles. Based on particle count measurements using a hemocytometer, at a concentration of, for example, 200 ppm, there were 26×10^6 calcium carbonate particles/ml but only 8.36×10^6 barite particles/ml. This difference in density, coupled with the observed similarity in response patterns for these two particulates when concentrations were prepared on a weight basis, suggests that the mechanism of a scallop's response to particulate material involves discrimination of particle weight rather than

particle number. The density of lignosulphonate was not obtained.

The third conclusion, which was alluded to above, is that whole drilling mud has a different effect than its major component in "pure" form (barite), and both other particulates tested (lignosulphonate, calcium carbonate). This is not an unprecedented result and there are several reasonable explanations for it. As mentioned earlier, Thompson and Bright (1977) found that various corals were unable to clear whole mud settling on their epidermis but could clear equal amounts of barite, calcium carbonate and aquagel. Such a difference could be due to synergistic interactions of the various constituents in whole mud or to the one or more constituents of whole mud that were not tested separately. There was no evidence that a biocide was being added to the mud at the time the test sample was obtained, but, as mentioned earlier, diesel fuel had apparently been added. It would be interesting to test the effect of diesel fuel on the scallop valve movement bioassay. Finally, the greater effect of mud could be due to any chemical changes that might occur when it is heated to temperatures as high as those that exist at a drill hole's bottom.

Section 4: A Submersible Monitoring Unit for the Behavior of Scallops and Other Bivalves.

Accompanying the increased presence of pollutants in our environment is the increased need for regulatory agencies to monitor these environments. In the past, such monitoring has primarily consisted of water chemistry measurements and descriptions of samples of the biota. More recently, the value of behavioral studies in evaluating sub-lethal effects of pollutants, in both the field and laboratory, has become increasingly recognized.

Monitoring the behavior of an animal in the field is a useful capability not only because it can reveal whether the animal is physiologically stressed, but also because it can reveal whether the animal is doing anything that might change the probability of it being exposed to a pollutant. This consideration is especially important in bivalved molluscs, for example, because they are able to close the valves for considerable periods of time. Where bivalves are used in field studies of bioaccumulation or depuration of pollutants, it would be valuable to know the amounts of time they were closed and open.

The prototype submersible monitoring unit (SMU) described here was originally developed for use with scallops, with the idea that a series of such units might be deployed in a circular array around an active drilling rig to monitor discharges of drilling muds and other materials. The SMU could easily be used with other bivalves in other environments. For

example, it could be used to insure that oysters were gaped (and hence exposed to the surrounding water) during a period of exposure to effluent from a sewage outfall.

The SMU is completely self-contained. Data are stored on magnetic tape using a recorder housed in the unit and battery power is also included. As currently designed, the SMU records only the valve movements of a single scallop. However, at the end of this section, there are a series of suggested modifications for the SMU which would extend its capabilities. With little additional cost, six or more scallops could be monitored by a single SMU. The water pumping sensor (Section 2) could also be added.

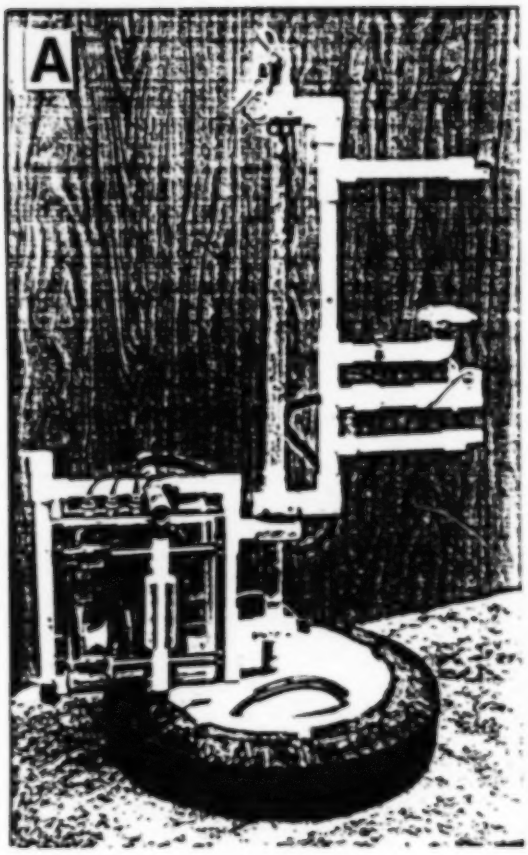
BASIC FRAMEWORK AND COMPONENTS

A 1 m tall vertical mast of 1.9 cm (I.D.) galvanized pipe is embedded in a concrete-filled tire (50 cm O.D.). Two rubber coated handles are also embedded in the concrete to facilitate handling the unit which weighs about 60 kg in air. Adjustable sleeve fittings on the mast permit attachment of the circuit housing and the monitoring frame. A galvanized steel shackle is bolted to the top of the mast and a swivel connects this shackle to sufficient 227-kg line to reach a surface buoy. A photograph of the prototype SMU is shown in Figure 4.1A.

MONITORING FRAME

The monitoring frame is basically identical to that designed for and used in laboratory studies (see Sections 2 and 3, respectively). However, the junction box is filled with plastic resin which hardens to a waterproof and pressure-resistant medium for greater isolation of the electrical connections. Also, PVC pipe elbows and extensions at the top and bottom of

Figure 4.1. Photographs of the entire SMU (A), the circuit box sitting in its support basket (B), the monitoring frame holding a scallop (C) and the magnetic tape recorder with the monitoring circuit board (D). In photograph C, the loop of nylon line has been adjusted so that the steel slug (right side of loop just beneath point where the clevis attached to the scallop is connected to the line) has been raised above the sensor coil below it. During normal operation, the slug would be inside the plastic tube around which the coil was wound, and consequently out of view.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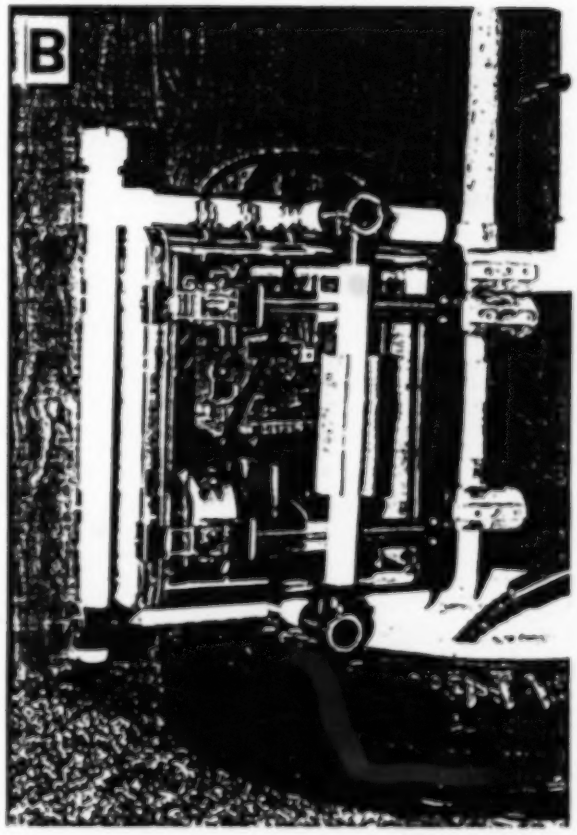
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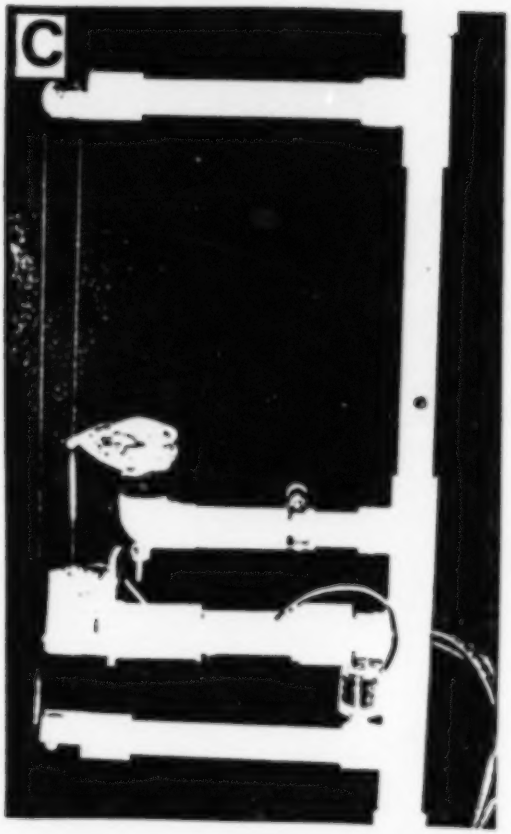
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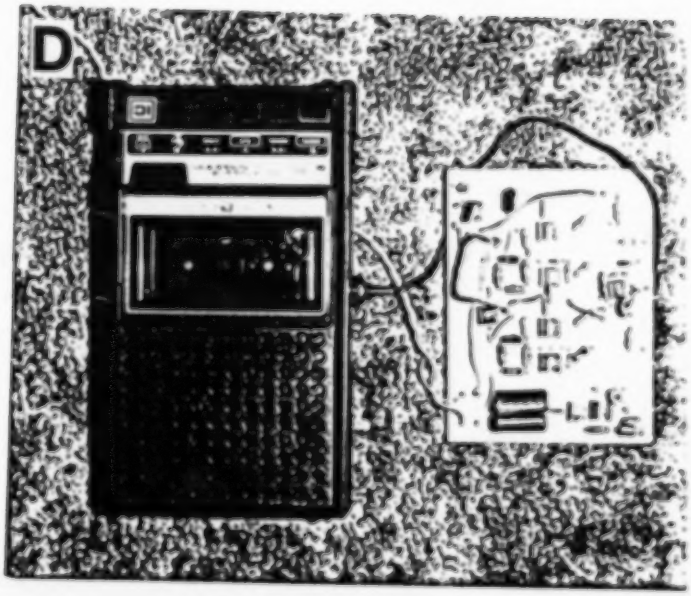
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the frame are used to mate it securely to the sleeves on the mast. Three 50 cm lengths of neoprene-coated cable (.6 cm diameter) connect the monitoring frame to the circuit housing. Photographs of the monitoring frame are shown in Figure 4.1A,C.

CIRCUIT HOUSING

The circuit housing (Ikelite #5910) is made of 1.3 cm thick injection-molded plexiglass and has a 1.9 cm thick cover with an O-ring seal; its inside dimensions are 12.7 x 22.9 x 20.3 cm. The manufacturer guarantees it against leakage to beyond 107 m depth. Permanent stainless steel bulkhead fittings (Ikelite #4007) were installed to accept the cables from the monitoring frame. A basket of 1.3 cm (I.D.) PVC pipe holds the housing securely to the sleeves on the mast. Photographs of the circuit housing are shown in Figure 4.1A,B.

A block diagram showing the major components contained in the circuit housing is shown in Figure 4.2. All batteries shown are 9 vdc nominal. A 30-minute timer controls the power for the tape recorder so that the SMU can be sealed and set down at a site, and the test animal has time to acclimate to conditions, before data recording starts. The regulators maintain their voltages at prescribed levels over a range of battery strengths. The monitoring circuit is identical to that shown in Figures 2.2 and 2.3 and the voltage-to-frequency converter circuit is identical to that shown in Figure 2.4.

The tape cassette in the recorder I used (a modified version of GE 3-5001) lasts 60 minutes. After the data are recorded, and the SMU and tape are retrieved, the audio output is fed to the frequency-to-voltage converter described in Section 2. The resulting output voltage can then be

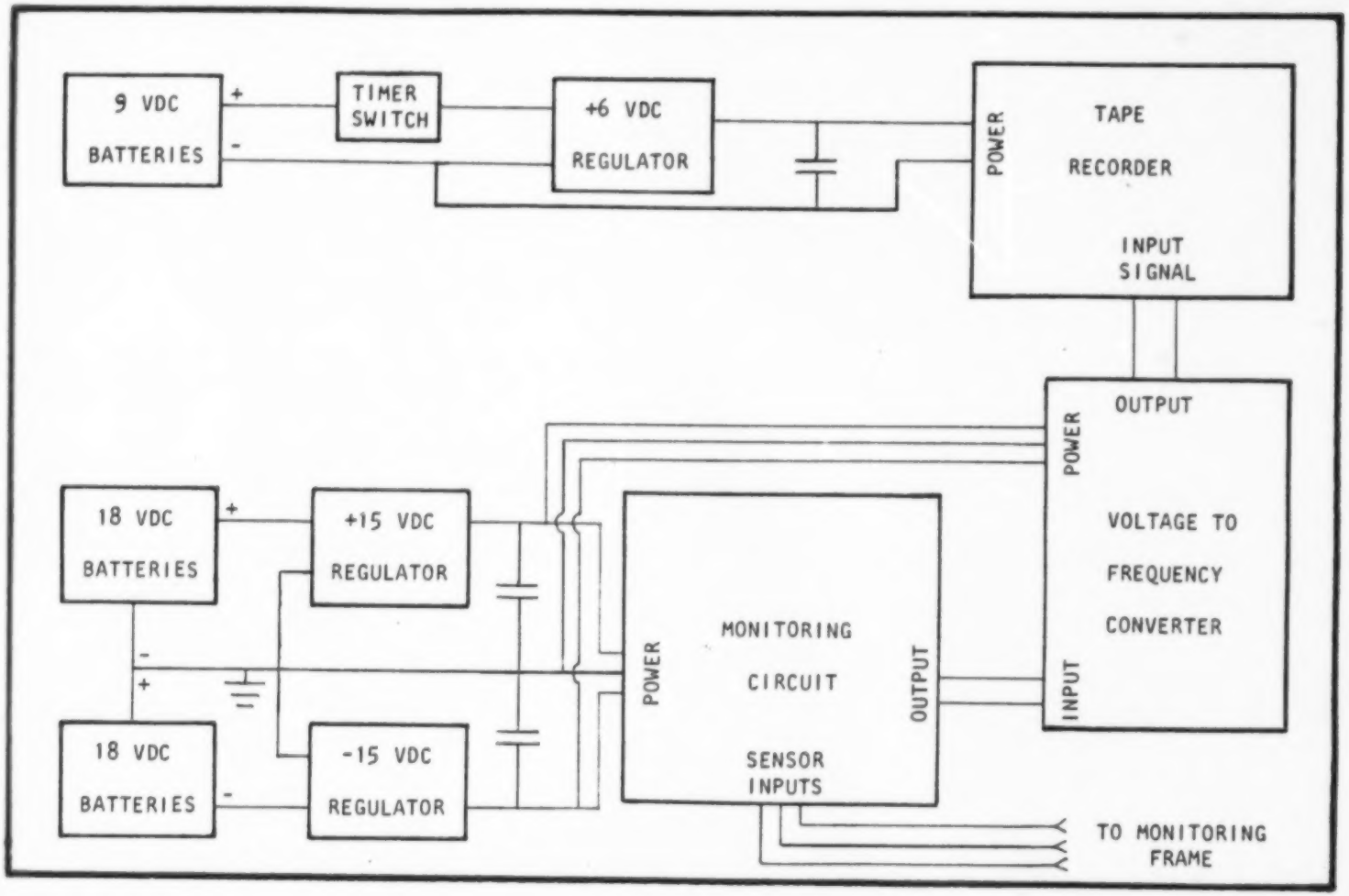

Figure 4.2. Block diagram showing the components contained in the circuit housing of the SMU. The manufacturer reports the box can withstand pressures to 106 m of depth. The monitoring frame is connected to the box by the waterproof cables and bulkhead connectors. The three capacitors are 100 μF.

fed to a conventional chart recorder (I used a Houston Instruments OMNISCRIBE B-5000) to obtain a visual representation of the changes in the test animal's valve movements over time. A photograph of the recorder and printed circuit board is shown in Figure 4.1D.

SAMPLE DATA

I have tested the SMU in Santa Rosa Sound, Escambia County, Florida. Salinity ranged from 20-25 ppt and water temperature from 11-14°C. Initial tests revealed thermally-caused drift and instability in the recorded signal. This was subsequently remedied by a circuit modification. Thereafter I observed a stable signal with only slight drift (down to 5°C, when the SMU was tested in a laboratory coldroom).

Several sections of a 60 minute record obtained from a scallop monitored at a depth of 2 meters in the field are shown in Figure 4.3. These data were originally recorded on magnetic tape in the SMU and were later portrayed on a chart recorded in the laboratory following the procedure described above. In both segments, there is evidence of the valves re-opening in a step-wise or "staircase" fashion, a situation also noted by Thomas and Gruffydd (1971) for Pecten maximus. Closure of the valves was much more rapid. Also in both segments, the scallop maintained a wider shell gape after the period of relatively rapid shell movements than it maintained before this period.

POSSIBLE MODIFICATIONS OF THE SMU

1. Often it would be desirable to operate an SMU for one or two weeks in the field without having to service it. The following modifications of the prototype would extend its operational capability:

A. The prototype SMU was powered by series and parallel combinations of disposable 9 vdc alkaline batteries (.5 amp-hour). The SMU would operate

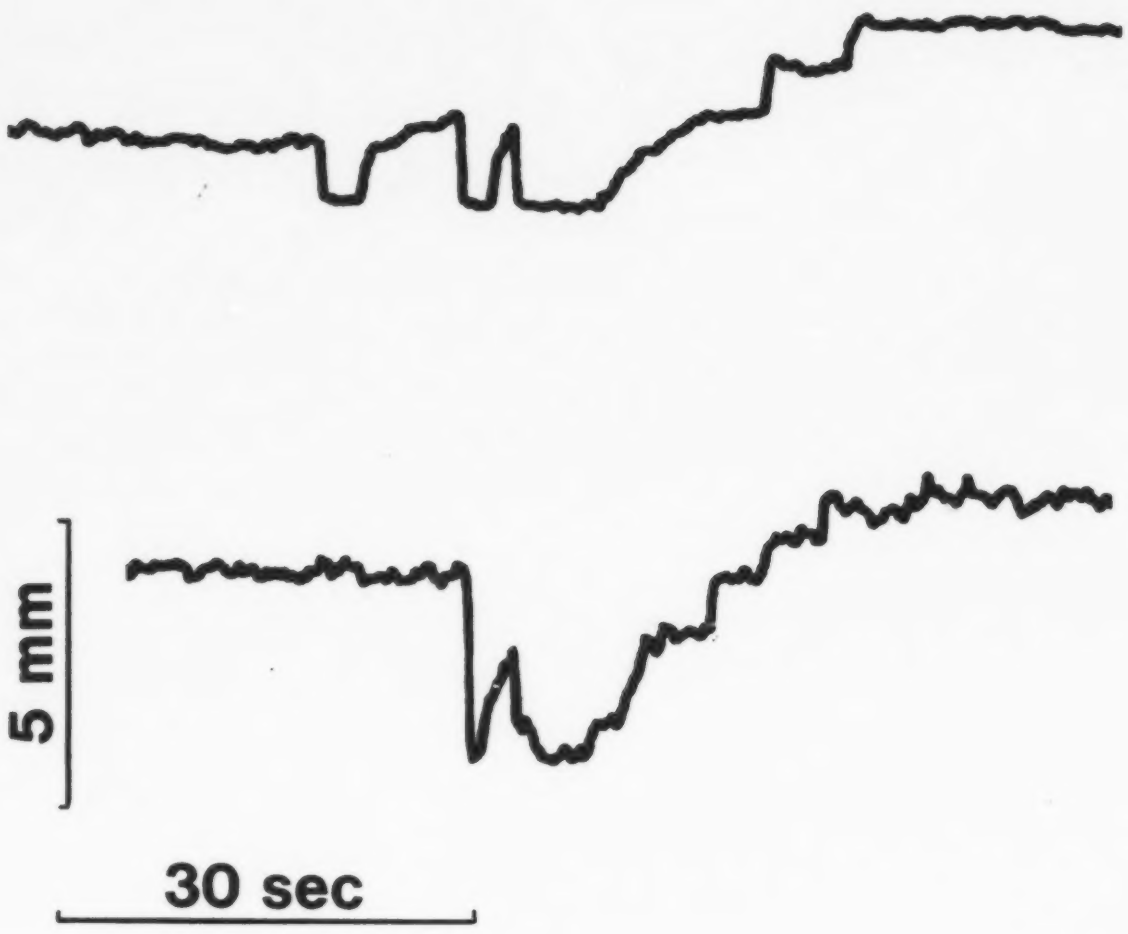

Figure 4.3. Two segments of a 60 minute record of valve movements made by the scallop Argopecten irradians. The scallop was held at 2 m depth in Santa Rosa Sound and its movements were recorded by the SMU. The data were recorded on magnetic tape within the SMU, and the tape was later 'read' in the laboratory to obtain the record. An approximate scale for gape width is given.

for about 90 minutes on one set of batteries. Batteries that are rechargeable and that have a higher amp-hour rating than the ones we used would improve the SMU's capabilities. Sealed 'Gel-type' rechargeable batteries are available and, although the initial investment would be significant (probably about $200 for the batteries and $125 for the additional housing that would be necessary/SMU), these would seem to be the best suited. Standard voltages are 6 and 12 vdc, so if the circuits could be adjusted to operate on 12 vdc rather than the present 15 vdc, they would be directly compatible.

B. Our magnetic tape recorder operated at the standard casette speed of 1.875 ips (=4.76 cm/sec) and the longest lasting (=thinest) magnetic tape permitted 60 minutes of continuous recording. Slower tape speeds could be achieved by altering the pulley size ratio or capstan diameter of the tape recorder.

C. Continuous records of behavior may not be appropriate or necessary for many applications. It might be desirable, for example, to record behavior for only a 5-minute period at the beginning of every hour. A simple microprocessor circuit could be added to the SMU which would turn on the monitoring circuit and tape recorder for only the 5-minute sampling periods.

2. The prototype SMU records the valve movements of only a single bivalve. A simple microprocessor circuit could be added to the SMU which switched a single monitoring circuit through a sequence of several sensor coils, each on a separate monitoring frame containing one bivalve. Thus, the behavior of a number of animals could be monitored, but not more than one at one time. The microprocessor could be combined with the microprocessor described above (1C) to achieve almost any sampling regime desired.

3. Since considerable time and money would be invested in an SMU, a backup method of locating and/or retrieving it (should the SMU-to-surface buoy cable break) might be attractive. For this purpose, an emergency disconnect could be installed near the base of the mast with control being via a sonic pinger. Once the mast became detached from the concrete base, the natural buoyancy of the circuit housing would bring the entire mast and attached components to the surface. Alternately, a standard sonic pinger might be attached to the SMU and its position could be located by a diver using a hand-held receiver-hydrophone.

4. An additional investment of about $50 (for parts) would permit building a circuit that would modify the analog signal coming from the monitoring circuits to a digital signal which could then be analyzed directly by a computer.

Section 5

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